children may however, have affected the clinical presentation. The youngest patient appeared to be growing well and to have normal endocrine function, but the older children manifested progressively more severe endocrine and growth problems. In Case 2 there was evidence that the growth rate was declining. In Case 3 measurements taken at the referring hospital indicated that growth velocity was probably normal between the ages of 2·7 and 3·3 years but that it diminished progressively thereafter. Case 4 "appeared to grow normally along the 25th percentile until the age of 1½ years. After this the growth curve flattened out, and she has hardly grown since the age of 3½." This raises the question whether there may be a progresssive disability—or whether lack of growth hormone may in fact not manifest itself immediately after birth.

The delayed onset of retarded growth in this series suggests that the main responsibility for early diagnosis rests in the interpretation of the ophthalmoscopic findings. The appearance of the disc, with its inner hypoplastic margin and an outer halo which probably represents the normal extent of the disc, is typical of the syndrome. Further experience suggests that there may be also be a paucity of retinal vessels. Fundoscopy and electrodiagnostic tests in blind children may suggest the syndrome, but confirmation can be obtained only by air encephalography. The characteristic findings are absence of the septum lucidum and dilatation of the chiasmatic cistern. Slender optic nerves may also be present, but we have not succeeded in showing the optic ventricle described by de Morsier[1] and demonstrated in one patient by Hoyt *et al.*[2]

Long-term follow-up of blind children with this syndrome is needed in order to make sure that they grow normally. We propose to treat the children of short stature in this series with growth hormone, and recent experience leads us to expect a satisfactory response.

References

1 de Morsier, G., *Schweizer Archiv für Neurologie und Psychiatrie*, 1956, 77, 267.
2 Hoyt, W. F., Kaplan, S. L., Grumbach, M. M., and Glaser, T. S., *Lancet*, 1970, 1, 893.
3 Tanner, J. M., Whitehouse, R. H., Hughes, P. C. R., and Vince, F. P., *Archives of Disease in Childhood*, 1971, 46, 745.
4 Ellenberger, C., and Runyan, T. E., *American Journal of Ophthalmology*, 1970, 70, 960.
5 Walton, D. S., and Robb, R. M., *Archives of Ophthalmology*, 1970, 70, 960.
6 Tanner, J. M., Whitehouse, R. H., and Takaishi, M., *Archives of Disease in Childhood*, 1966, 41, 454, 513.
7 Harden, A., and Pampiglione, G., *Lancet*, 1970, 1, 805.
8 Jackson, D., Grant, D. B., and Clayton, B. E., *Lancet*, 1968, 2, 13.
9 Barnes, N. D., Joseph, J. M., Atherden, S. M., and Clayton, B. E., *Archives of Disease in Childhood*, 1972, 47, 66.

For Debate . . .

Bile Acids: A pH Dependent Antibacterial System in the Gut?

I. W. PERCY-ROBB, J. G. COLLEE

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Summary

Bile acids are secreted in the bile in the form of conjugates and many species of intestinal bacteria can rapidly deconjugate them. Studies have shown that an unconjugated bile acid may have bactericidal and bacteriostatic effects, which are pH dependent. It is proposed that unconjugated bile acids may be involved in a homoeostatic mechanism, preventing bacterial growth in the small intestine.

Introduction

The small intestine has a recognized bacterial flora that is both qualitatively and quantitatively different from the flora of the caecum and colon[1]. Investigation of the mechanisms that limit bacterial proliferation in the bowel has recently stimulated interest because the functional consequences of bacterial overgrowth in the small intestine are known to cause symptoms associated with malabsorption of fat[2–4] and of vitamin B_{12}[5] and abnormalities of protein metabolism.[6] We report a bactericidal effect observed when strains of *Clostridium welchii* or of *Bacteroides* spp., some of which were obtained from human faeces, were grown in the presence of the bile salt taurocholic acid, a normal component of human bile. Normal faeces of healthy adults in Britain contain about 10^4 *Cl. welchii* and about 10^{10} *Bacteroides* spp./g wet weight.[7]

University Department of Clinical Chemistry, Royal Infirmary, Edinburgh
I. W. PERCY-ROBB, M.B., PH.D., Senior Lecturer in Clinical Chemistry

Department of Bacteriology, University Medical School, Edinburgh
J. G. COLLEE, M.D., M.R.C.PATH., Reader in Bacteriology

Methods

Strains of *Cl. welchii* or of *Bacteroides* spp. (Table I) were grown in fluid media (Oxoid Brewer medium or Oxoid nutrient broth with glucose 0·5%) in anaerobic jars containing hydrogen with 10% CO_2. Brewer medium contains 0·5 g glucose/100 ml. In most experiments sodium taurocholate was added to the medium at a concentration of 4 mmol/l. Parallel cultures were set up in medium without taurocholate. The effect of cholic acid (the product of bacterial deconjugation of taurocholate) was

TABLE I—*Test Strains of Bacteria**

Strain	Description
Cl. welchii:	
L2A	A classical type-A strain (reference strain)
C24	β-haemolytic, from human ileostomy fluid
CX	β-haemolytic, from human faeces
N.C.T.C.8359	A typical food-poisoning strain, Hobbs type 1
Bacteroides spp.:	
N.C.T.C.7155	*B. necrophorus* (reference strain)
N.C.T.C.9343	*B. fragilis* (reference strain)
G.N.A.B.3	From an infected wound
G.N.A.B.7	From infected wound
G.N.A.B.9	From human faeces
G.N.A.B.10	From human faeces

*N.C.T.C. = National Collection of Type Cultures; G.N.A.B. = Gram-negative anaerobic bacillus.

studied by adding cholic acid in graded amounts to parallel series of bijou bottles of horse-flesh broth[8] that had been adjusted to pH values of 5·8, 6·4, and 7·2. The media were sterilized by autoclaving at 121°C for 20 minutes and, after cooling, were seeded with standard volumes (0·02 ml) of the test strain grown in a cooked-meat broth culture. Total cell counts were determined in a counting chamber and viable counts by spreading standard volumes of serial dilutions on horse blood agar incubated under anaerobic conditions with 10% CO_2.

Deconjugation of taurocholate was assessed by absorption chromatography on thin layers of silica gel G with the solvent system described by Kelly and Doisy.[9] The concentration of unconjugated cholic acid was measured by quantitative densitometry of thin-layer chromatograms.[10] Cholic acid and taurocholate (sodium salt) were obtained from Maybridge Chemical Co., Tintagel, Cornwall, and 24^{14}C-taurocholate was obtained from Tracerlab Ltd., Ship Yard, Weybridge, Surrey.

Results

Deconjugation of Taurocholate.—When cultures containing taurocholate were incubated for 48 hours complete deconjugation occurred consistently with each of the strains of *Cl. welchii* and varied between 50 and 100% with the *Bacteroides* spp. This finding was shown both with Brewer medium and in nutrient broth enriched with glucose. In radioactive tracer studies there was no evidence that the cholic acid released from taurocholate was further degraded.

Observations on Bacterial Growth in Presence of Taurocholate.—Graphs of the total cell counts showed classical growth curves; maximum counts of about 10^8 organisms/ml were reached after 12 hours. The presence of taurocholate had no effect on the total counts. The viable counts in cultures containing taurocholate showed almost complete death after 24 hours, whereas the viability of the organisms in taurocholate-free cultures at that stage was 100% (Fig. 1).

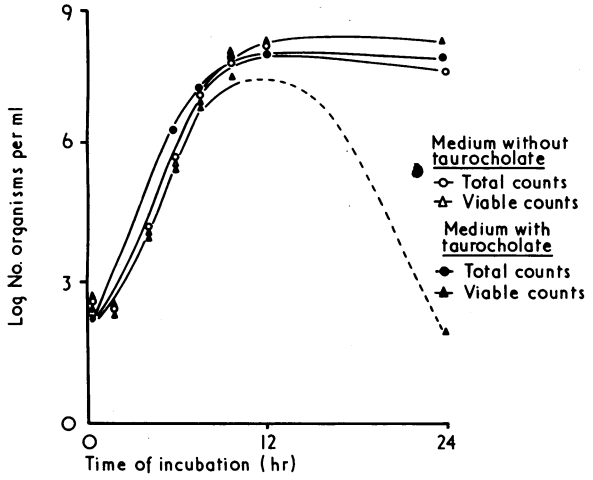

FIG. 1—Total cell counts and viable counts of strain of *Cl. welchii* grown in Brewer medium with and without taurocholate (4 mmol/l.).

Changes in pH during Bacterial Growth.—The pH of a glucose-free control culture did not change significantly (Fig. 2). *Cl. welchii* fermented glucose in the test media and the final pH was lowest (pH 4·6) in those cultures that did not contain taurocholate. In the test cultures in which deconjugation occurred the final pH values were 5·5 and 5·8. The bactericidal effect observed in the test cultures was therefore not directly attributable to a fall in pH alone.

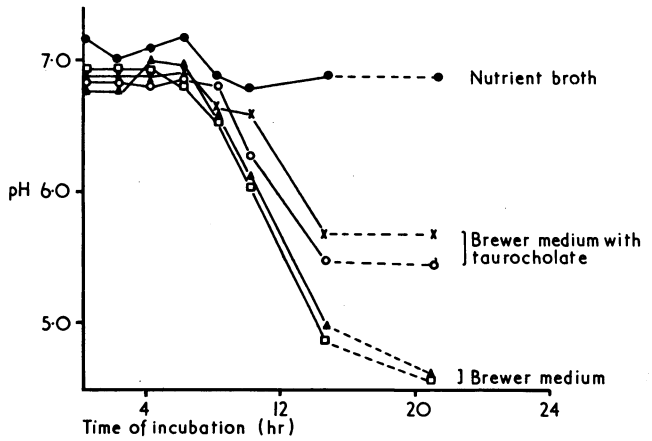

FIG. 2—pH values of cultures of two strains of *Cl. welchii* grown in Brewer medium with and without taurocholate in comparison with those observed with a strain grown in nutrient broth.

Cholic Acid Production during Bacterial Growth.—As a result of the deconjugation of taurocholate, cholic acid was clearly demonstrable in the test cultures after 8-10 hours, and thereafter the concentration increased (Fig. 3).

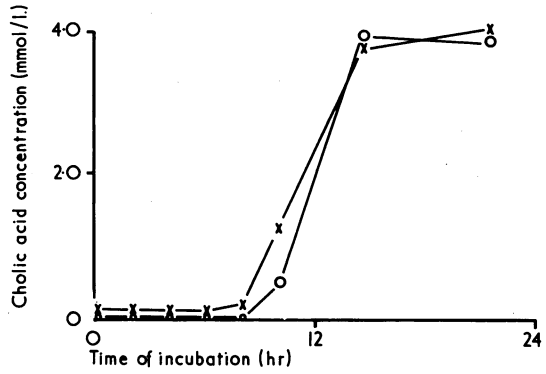

FIG. 3—Release of cholic acid by two strains of *Cl. welchii* grown in Brewer medium containing taurocholate (4 mmol/l.).

TABLE II—*Growth of* Cl. welchii *and* Bacteroides *spp. in Presence of Graded Concentrations of Cholic Acid at pH 5·8, 6·4, and 7·2*

Test Strain	pH of Test	Observed growth* at Stated Concentration of Cholic Acid (mmol/l.)													
		6		3		1·5		0·75		0·375		0·18		0	
		T.	G.	T.	G.	T.	G.	T.	G.	T.	G.	T.	G.	T.	G.
Cl. welchii C 24	5·8	—	—	—	—	—	—	+	++	+++	+++	+++	+++	+++	+++
	6·4	—	—	—	++	±	++	+	++	+++	+++	+++	+++	+++	+++
	7·2	±	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Cl. welchii N.C.T.C. 8359	5·8	—	—	—	—	—	—	—	+	±	+++	++	+++	++	+++
	6·4	—	—	—	—	—	+	—	+	++	+++	++	+++	+++	+++
	7·2	—	—	±	+++	+	+++	++	+++	+++	+++	+++	+++	+++	+++
B. fragilis N.C.T.C. 9343	5·8	—	—	—	±	—	+	—	+	+	+++	++	+++	++	+++
	6·4	—	±	—	±	+	++	+++	+++	+++	+++	+++	+++	+++	+++
	7·2	—	++	+	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Bacteroides sp. G.N.A.B. 10	5·8	—	—	—	—	—	—	—	++	—	+++	—	+++	±	++
	6·4	—	—	—	+	—	++	+	+++	+	+++	++	+++	+++	+++
	7·2	—	++	±	+++	+	+++	++	+++	++	+++	+++	+++	+++	+++

*T. = Turbidity in anaerobic broth.
G. = Growth on subsequent anaerobic subculture on blood agar.

Antibacterial Effect of Cholic Acid.—It seemed likely that the loss of viability that paralleled deconjugation was attributable to an antibacterial effect of cholic acid; the minimum inhibitory concentration of cholic acid was therefore studied. The observed changes in pH in the test cultures led us to perform the tests of minimum inhibitory concentration at various pH values for each of the four test strains of *Cl. welchii*. These studies were extended to include the six strains of *Bacteroides* spp. A bactericidal effect of cholic acid was shown with each of the test strains; representative results are given in Table II. The antibacterial effects were greatest at a pH of 5·8 and least at a pH of 7·2.

Discussion

The extent to which the host's gastrointestinal secretions may impair bacterial growth in the gut remains uncertain. Saliva inhibits the growth of some bacteria and may support the growth of others.[11] A relation between the bactericidal effects of gastric and duodenal juice and the secretion of hydrogen ions by the stomach was shown by Arnold and Brody[12], and the death of vegetative cells of *Cl. welchii* when exposed to aqueous solutions at pH 1·5-2·6 has recently been confirmed,[13] though the length of exposure required to produce the bactericidal effect in vitro is perhaps unlikely to be achieved regularly in vivo. Meynell[14] considered that the growth of *Staphylococcus aureus* given by mouth to mice is inhibited primarily as a result of volatile fatty acids in normal caecal contents. Bergeim[15] attributed the toxicity of extracts of human faeces for yeasts and *Escherichia coli* to the presence of volatile fatty acids, mainly formic, acetic, and butyric acid. He emphasized that the antimicrobial effect of these acids was not due to acidity alone. The toxic effects were related to the dissociated molecule and were therefore observed at pH values less than the dissociation constants of the volatile acids.

Many bacteria isolated from both small intestine and colon can deconjugate each of the bile acid conjugates secreted in bile[16] to yield the free acid. While it is well known that conjugated bile acids—for example taurocholate—inhibit the growth of some organisms in vitro, it has been shown by Floch *et al.*[17] that bile-tolerant intestinal anaerobes may be inhibited by unconjugated bile acids such as cholic and deoxycholic acid. The growth-inhibitory effects were most evident with *Bacteroides* and *Lactobacillus* spp., whereas coliform organisms were not inhibited. These workers gave details of the antibacterial effects of bile acids at concentrations in the range 1-25 mg/ml—that is, 2·5-62·5 mmol/l.—and the pH of the test system during growth was not reported. They found that consistent inhibition occurred only when the concentration of bile acid was "0·5-1·0%"—that is, 12·5-25 mmol/l. As the concentration of free bile acids in the terminal ileum is approximately 1·0 mmol/l.,[18] it is now important to examine the sensitivity of bacteria to concentrations in this range and to bear in mind the pH of the system.

The pronounced bactericidal effect that we have described[19] with cultures of *Cl. welchii* in Brewer medium supplemented with taurocholate occurred simultaneously with the production of demonstrable amounts of unconjugated cholic acid in the medium and with a fall of pH to 5·6. That the fall of pH alone was not directly bactericidal is clearly evident from the results obtained with control cultures devoid of taurocholate; the viability of these cultures was maintained (Table II). The results of our subsequent tests showed that all of our test strains of *Cl. welchii* and *Bacteroides* spp. were highly sensitive to cholic acid at pH 5·8 and successively less so at pH 6·4 and 7·2. In general a bactericidal effect was clearly shown with cholic acid at a concentration of 1·5 mmol/l., provided that the pH was 5·8. At the higher pH values a decreasing degree of sensitivity was found. In preliminary studies with deoxycholic and chenodeoxycholic acids we showed a bactericidal effect with concentrations of the order of 0·2-0·4 mmol/l. at pH 5·8, and bacteriostatic effects were shown with these acids at lower concentrations.

As bile salts present in the small intestine are largely reabsorbed in the terminal ileum, any postulated local effect of bile acids in the lumen of the normal intestine is more likely to occur in the small gut. The primary role of the ileocaecal valve in compartmenting the bacterial flora of the upper and lower gut may now be challenged. Gorbach *et al.*[1] showed that a bacterial flora consisting of a modified faecal flora is present in the terminal ileum. Northfield and colleagues[18] showed free bile acids in the terminal ileum of man. If the antibacterial effect noted in the present study operates in vivo it is tempting to suggest that free bile acids at that site are involved in a homoeostatic mechanism and prevent retrograde bacterial colonization in the upper gut.

In the colon the further degradation of the unconjugated bile acids produces a new series of (secondary) bile acids, some of which are reabsorbed and reappear in bile. In patients who have a resection of the terminal ileum the normal absorptive site for bile acids is reduced and abnormally large amounts pass into the large bowel. Secondary bile acids are not present in the bile of these patients.[20] The direct antibacterial effect of cholic acid that we have observed may thus extend to the large gut in patients with terminal ileal resection and there operate against bacterial systems that would normally degrade bile acids.

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References

1 Gorbach, S. L., Plaut, A. G., Nahas, L., and Weinstein, L., *Gastroenterology*, 1967, **53**, 856.
2 Tabaqchali, S., and Booth, C. C., *Lancet*, 1966, **2**, 12.
3 Drasar, B. S., and Shiner, M., *Gut*, 1969, **10**, 812.
4 Gorbach, S. L., and Tabaqchali, S., *Gut*, 1969, **10**, 963.
5 Dellipiani, A. W., and Girdwood, R. H., *Clinical Science*, 1964, **26**, 359.
6 Jones, E. A., Craigie, A., Tavill, A. S., Franglen, G., and Rosenoer, V. M., *Gut*, 1968, **9**, 187.
7 Hill, M. J., *et al.*, *Lancet*, 1971, **1**, 95.
8 Cruickshank, R., *Medical Microbiology*, 11th edn., p. 744. Edinburgh, Livingstone, 1968.
9 Kelly, R. L., and Doisy, E. A., jun., *Federation Proceedings*, 1964, **23**, 173.
10 O'Moore, R. R. L., and Percy-Robb, I. W. In preparation.
11 Williams, N. B., and Dowlen, D. O., *Archives of Oral Biology*, 1959, **1**, 48.
12 Arnold, L., and Brody, L., *American Journal of Hygiene*, 1926, **6**, 672.
13 Sutton, R. G. A., and Hobbs, B. C., *Journal of Medical Microbiology*, 1971 **4**, 539.
14 Meynell, G. G., *British Journal of Experimental Pathology*, 1963, **44**, 625.
15 Bergeim, O. *Journal of Infectious Diseases*, 1940, **66**, 222.
16 Shimada, K., Bricknell, K. S., and Finegold, S. M., *Journal of Infectious Diseases*, 1969, **119**, 273.
17 Floch, M. H., Gershengoren, W., Elliott, Sylvia, and Spiro, H. M., *Gastroenterology*, 1971, **61**, 228.
18 Northfield, T. C., Condillac, E., and McColl, I., *Gut*, 1970, **11**, 1063.
19 Percy-Robb, I. W., Miller, B. R., and Collee, J. G., *Journal of Medical Microbiology*, 1972, **5**, P. viii.
20 McLeod, G. M., and Wiggins, H. S., *Lancet*, 1968, **1**, 873.